To identify activators, which generally act as positive gating modulators, a lower Ca^{2+} concentration (~200 nM) was used. The QPatch results were benchmarked against manual patch-clamp electrophysiology by determining the potency of several commonly used KCa3.1 inhibitors (TRAM-34, NS6180, ChTX) and activators (EBIO, riluzole, SKA-31). Collectively, our results demonstrate that the QPatch provides a comparable but much faster approach to study compound interactions with KCa3.1 channels in a robust and reliable assay.

Supported by U54NS079202, R21NS072585 and T32-GM008799 from NIH.

2747-Pos Board B439

Diversity in the Pharmacological Profile of Heterotetrameric K_V2/K_VS Channels for Channel Blockers

Jeroen I. Stas, Elke Bocksteins, Alain J. Labro, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.

Voltage-gated K^+ (Kv) are tetramers of α-subunits each consisting of 6 transmembrane segments (S1-S6) and a cytoplasmic N- and C-terminus. The S5-S6 segments of each subunit assemble to generate the central pore while the S1-S4 segments form the voltage-sensing domains. The PXP motif in the middle of S6 provides a degree of flexibility to the bottom half of the S6 segment which is necessary for channel gating. This region is also critical for the interaction with channel blockers. Based on sequence homology, eight *Shaker*-related Kv subfamilies have been identified: Kv1-Kv6, Kv8-Kv9. The silent (KvS) subunits (Kv5-Kv9) cannot form homotetramers but assemble with Kv2 subunits into Kv2/KvS heterotetramers that display unique biophysical properties. KvS subunits lack the 2nd proline residue of the PXP motif which may impact on the pharmacological profile of channel blockers. We tested this hypothesis by using the Kv1.5(P511G) mutant in which the 2nd proline of the PXP motif was replaced by a glycine. Homotetrameric Kv1.5(P511G) channels were insensitive to 4-AP while heterotetrameric Kv1.5-Kv1.5(P511G) channels (stoichiometry controlled by using dimers), still displayed current inhibition. However, Kv1.5-Kv1.5(P511G) channels were significantly less sensitive displaying an IC_{50} values of 16 mM instead of 270μM for wild type (WT) Kv1.5. Similarly, heterotetrameric Kv2/KvS channels displayed an altered affinity for 4-AP compared to WT Kv2.1; 18 mM (IC_{50} for Kv2.1) inhibited 17%, 60%, 82% and 13% of Kv5.1, Kv6.3, Kv8.1 and Kv9.3-containing currents, respectively. Furthermore, the heterotetrameric Kv2/KvS channels displayed also a subtle change in the affinity for the open channel blockers quinidine and flecainide. These results suggest that the absence of a complete PXP motif in one or two out of four subunits alters the pharmacological profile. (Supported by FWO fellowships to JS and EB & grant FWO-G.0449.11N to DJS).

2748-Pos Board B440

Subunit Composition Determines Gβγ Activation of Single Girk Channels

Daniel Yakubovich[1,2], Nathan Dascal[1].
[1]Tel-Aviv University, Sackler School of medicine, Tel-Aviv, Ramat Aviv, Israel, [2]The Edmond and Lily Safra Children's Hospital, Ramat-Gan, Israel.

GIRK (G-protein activated inward rectifier potassium channels) are direct effectors of Gβγ expressed in cardiac myocytes, neurons and other cells. GIRKs modulate resting membrane potential and mediate inhibitory actions of many neurotransmitters. GIRKs are involved in heart rate inhibition by vagus nerve, long term potentiation, pain mediation, drug addiction, etc. These channels are tetramers and GIRK1/2 is most abundant in brain while GIRK1/4 is mainly expressed in cardiomyocytes. We compared the kinetic single-channel properties of GIRK1/2 and GIRK1/4. Channels were expressed in Xenopus leaves oocytes with Gβγ subunit of G-proteins. The dose of Gβγ was chosen to produce maximal activation, as verified in whole-cell recordings. GIRK channels in cells co-expressing Gβγ demonstrated robust activity in cell-attached membrane patches. Recordings demonstrating no overlaps for long episodes of activity (~ 5-10 min) were considered as single channel and were subjected to detailed kinetic analysis. We found that maximal open probability ($P_{o,max}$) of GIRK1/4 ($0.05 \pm .01$, n = 5) is almost 3 fold lower than that of GIRK1/2 (0.15 ± 0.027, n= 6). Mean closed time of GIRK1/4 (35.88 ± 6.6 ms, n = 5) was significantly longer than of GIRK1/2 (14.74 ± 3.58 ms, n = 6). No significant difference was found between the mean open times. Closed times distribution of GIRK1/4 was satisfactorily fitted with 5 exponents (as found previously in excised patches), whereas only 4 exponents were sufficient for fitting the closed times distribution of GIRK1/2. It was hypothesized that GIRK channel as a tetramer can be found in 5 closed states (0-4 bound Gβγ). The fact that only 4 closed sates are populated in GIRK1/2 can point to higher affinity to Gβγ compared to GIRK1/4. Analysis of published literature supports lower EC_{50} values for GIRK1/2 than for GIRK1/4 observed in dose-response to Gβγ in excised patches.

2749-Pos Board B441

G-Protein Activated Inwardly Rectifying Potassium Channels Control Motility of Breast Cancer Cells

Simin Rezania[1], Chouyang Li[1], Sarah Kammerer[1], Astrid Gorischek[1], Trevor Devaney[1], Amir Hassan Zarnani[2], Thomas Bauernhofer[3], Wolfgang Schreibmayer[1].
[1]Department of Biophysics.Medical University of Graz, Graz, Austria, [2]Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [3]Oncology Department,Medical University of Graz, Graz, Austria.

Mortality of breast cancer is very high when it metastases to distant organs. New early tumor markers and mechanistic insight into carcinogenesis and metastasisation are needed to treat the disease at curable stages. GIRK channels are activated by GPCRs and regulate resting potential and excitability of neurons, myocytes and other cells. Recently GIRK proteins have been associated with endocrine adenomas and also breast cancer (Stringer et al.2001, Brevet et a.l 2008 and Choi et al.2009). In this study we analyzed the vital parameters of cancerous and noncancerous breast cell lines when human GIRK1 and splice variants (hG1a, hG1c, hG1d) are overexpressed or silenced.

GIRK1a, GIRK1c and GIRK1d were stably overexpressed and knocked out in breast cell lines MCF7, MDA-MB-231 and MCF 10A. Vital parameters including invasion, wound healing, adhesion, proliferation and motility were evaluated.

Our findings revealed that overexpression of GIRK1a significantly increased the velocity 0.102 ± 0.035 (μm/min) in overexpresssed vs. 0.05 ± 0.02 in vector control cells ($P<0.001$) and motility coefficient 0.2 ± 0.3 (μm2/min) in overexpresssed vs. 0.05 ± 0.06 in vector control cells ($P<0.001$) of the MCF7 cell lines. Overexpression of hG1c in MCF7 significantly increased invasiveness of the cells ($P<0.001$) as measured by matrigel assay. Overexpression of the GIRK1 and splice variants didn't have significant effect on proliferation, wound healing and adhesion of the MCF7 cells.

Stably overexpression of GIRK1 and splice variants in MCF7 cause increased velocity, motility coefficient and invasion of the cells. Expression of GIRK might be a new diagnostic biomarker of breast cancer and possibly is causally involved in metastasisation.

Acknowledgements: The research is funded by the Austrian Science Foundation (FWF; P22974-B19 to W. S and KLIF 182 to T. B).

2750-Pos Board B442

Maximal Activity of KcsA, Kirbac1.1 and Kir2.1 Channels are Differentially Regulated by Membrane Thickness

Benoit Mondou, Louis Sasseville, Jean-Louis Schwartz, Jurgen Sygusch, Nazzareno D'Avanzo.
Biochemistry, Université de Montréal, Montreal, QC, Canada.

Ion channels may be regulated by numerous factors, including the physiochemical properties of the membrane in which they are imbedded. Hydrophobic matching between the hydrophobic thickness of the bilayer and the channel's hydrophobic length is thought to minimize the energetic penalty that would be needed to solve hydrophobic residues or exposed lipid tails. Here we examine the role of hydrophobic matching in regulating the activity of 3 potassium channels, KcsA, KirBac1.1 and human Kir2.1. Purified channels were reconstituted into membranes containing 25 mol% POPG and 75 mol% diC_n:1PC ($14 < n < 22$) (+ 1 mol% $PI(4,5)P_2$ for Kir2.1). $^{86}Rb^+$ influx assays indicate that KcsA channels are maximally active in thick membranes (diC20:1PC), while both KirBac1.1 and Kir2.1 channels were maximally active in thinner membranes (eg. < diC18:1PC). Single channel recordings in lipid bilayers of the same compositions indicate that membrane thickness affects the open probability of KcsA, but not unitary conductance. SAXS was used to quantify the hydrophobic thickness of each of our experimental conditions in order to quantify the energy associated with hydrophobic matching for these 3 proteins. Our initial calculations suggest the energies associated with membrane stretching or curvature are too great to account for hydrophobic matching in these channels, and suggest tilting of the α-helices of the proteins are required for these channels to be maximally active. Molecular dynamic simulations provide further insight into the molecular details associated with hydrophobic matching for each of these channels.

2751-Pos Board B443

Activation of Slack Channels Alters their Interactions with the Pp1 Targeting Protein Phactr1

Matthew R. Fleming[1], Leonard K. Kaczmarek[2].
[1]Pharmacology, Yale University, New Haven, CT, USA, [2]Pharmacology and Cellular and Molecular Physiology, Yale University, New Haven, CT, USA.

Slack Na^+-activated K^+ channels contribute to neuronal adaptation during sustained stimulation and regulate the temporal accuracy of action potentials.

The C-terminus of Slack interacts with the mRNA-binding protein FMRP (Fragile X Mental Retardation protein). Using yeast-two hybrid and co-immunopurification experiments we have established the Slack binds Phactr1, a Protein Phosphatase 1 targeting protein. This interaction likely regulates the modulation of Slack by protein kinase C (PKC), which increases current amplitude and slows the rate of activation rate by phosphorylation of a specific serine (S407) in the Slack cytoplasmic C-terminus. Using resonance wavelength grating optical biosensors, we have determined that phosphorylation of Slack at S407 by PKC produces a sustained decrease in mass near the plasma membrane. The decrease in mass requires the very C-terminal domain of Slack, previously shown to be required for Slack/FMRP interactions. To determine whether the decrease in mass results from the translocation of either FMRP or Phactr1 from the channel upon activation, RNAi was used to suppress expression of FMRP, Phactr1 and other potential partners. The presence of Phactr1, but not FMRP, was found selectively to be required to observe a decrease in mass. Gain-of-function human mutations in Slack channels that mimic constitutive phosphorylation at S407 cause malignant migrating partial seizures of infancy (MMPSI), an epileptic encephalopathy of infancy that combines seizures with severe developmental delay. Activation of PKC does not produce a change of mass in these mutant channels, suggesting that Phactr1 fails to associate/dissociate. Our results suggest that activation of Slack alters its interaction with Phactr1, which may influence the phosphorylation state of Slack, but also of associated proteins such as FMRP, providing a link between channel activation and downstream signaling pathway that regulate normal neuronal development and intellectual ability.

2752-Pos Board B444

Effects of E-LXA4 on Kv and Kir Recorded from Bone Marrow Mouse Macrophages

Alicia de la Cruz, Cristina Moreno, Alvaro Macias, Angela Prieto, Teresa Gonzalez, Carmen Valenzuela.
Instituto de Investigaciones Biomedicas CSIC-UAM, Madrid, Spain.

Macrophages may tune the immune response toward inflammation or tolerance. The proliferation, activation, and resolution or tolerance of immune cells is mainly modulated by membrane transduction of extracellular signals. Some of these interactions involve changes in transmembrane ion fluxes that, in turn, modulate the network of intracellular signaling. Potassium channels modulate macrophage physiology. Blockade of voltage dependent potassium channels (Kv) by specific antagonists decreases macrophage cytokine production and inhibits proliferation. Therefore, Kv channels have been proposed as anti-inflammatory targets. We studied the effects of 15-epi-lipoxin (e-LXA_4), an endogenous eicosanoid released in the presence of aspirin on early signaling and on voltage-dependent potassium (Kv: Kv1.3, Kv1.5) and inward rectifier potassium channels (Kir) in mice bone marrow-derived macrophages (BMDM) and in cultured HEK293 cells. Electrophysiological experiments recordings were performed by the whole-cell patch-clamp technique. Treatment of BMDM with e-LXA_4 inhibited LPS-dependent activation of NF-κB and IKKβ activity and protected against LPS activation-dependent apoptosis. Acute treatment of LPS-stimulated BMDM with e-LXA_4 resulted in a decrease of Kv currents, compatible with attenuation of the inflammatory response. More importantly, long-term treatment of LPS-stimulated BMDM with e-LXA_4 significantly reverted LPS effects on Kv and Kir currents. These effects were partially mediated via the lipoxin receptor (ALX), since were partially reverted in the presence of a selective ALX receptor antagonist. In the present work, we provide evidence for a new mechanism by which e-LXA_4 contributes to inflammation resolution consisting in the reversion of LPS effects on Kv and Kir currents in macrophages. In fact, the lack of effects on Kv and Kir currents recorded in HEK293 cells demonstrated that an intracellular signaling network is required to produce the effect observed in BMDM on the LPS-dependent activation with e-LXA_4. Granted by SAF2010-14916 and FIS-RIC RD12/0042/0019.

2753-Pos Board B445

Effects of Cl888 on Kv4.3, Kv4.3/Kchip2C and Kv4.3/KChiP3 Channels

Angela Prieto[1], Pilar Cercos[2], Alicia de la Cruz[1], Teresa Gonzalez[1], Marta Gutierrez-Rodriguez[2], Jose-Ramon Naranjo[3], Carmen Valenzuela[1].
[1]Instituto de Investigaciones Biomedicas CSIC-UAM, Madrid, Spain, [2]Instituto de Quimica Medica CSIC, Madrid, Spain, [3]Centro Nacional de Biotecnologia, Madrid, Spain.

Kv4.3 generates the transient outward current that plays an essential role in shaping the early phase of the cardiac action potential. These channels are regulated by a family of calcium-binding proteins called KChIPs. Four members of this family have been cloned. KChIP3 and KChIP2c bind to Kv4.3 in the endoplasmic reticulum and facilitate trafficking to the membrane and regulate the channel gating. CL888 induces alteration or disruption in the binding between KChIP1 and Kv4.3, modulating the function of the complex. The aim of our study was to determine the effects of CL888 on Kv4.3/KChIP3 and Kv4.3/KChIP2 complex, as well as on Kv4.3 alone. KChIP3, KChiP2 and Kv4.3 channels were expressed in CHO cells. Currents were recorded using the whole-cell patch-clamp technique. Block of Kv4.3/KChIP3 channels induced by CL888 was concentration dependent with an IC50 of 23 nM, whereas Kv4.3 and Kv4.3/KChIP2c was not. At 100 nM, degree of block ranged according to: Kv4.3/KChIP3>Kv4.3/KChIP2c>Kv4.3. In all 3 channel complexes, CL888 accelerated the inactivation kinetics of the current by decreasing the slow time constant (τs). Thus, for Kv4.3/KChIP3 the τs value changed from 20.6 ± 1.6 ms to 17.0 ± 1.5 ms (n=5, $P<0.05$); for Kv4.3, from 19.7 ± 1.6 ms to 10.8 ± 1.5 ms (n=4, $P<0.05$); and for Kv4.3/KChIP2, from 38.1 ± 5.8 ms to 23.9 ± 4.0 ms (n=5, $P<0.05$). CL888 did not affect the voltage dependency of steady-state inactivation of Kv4.3/KChIP3 channels. However, it accelerated their recovery from inactivation kinetics ($\tau re=62.0 \pm 12.1$ ms vs. 49.8 ± 9.8 ms, n=6, $P<0.05$). We conclude that CL888 binds to Kv4.3 channels and modulates the gating and the kinetics of these channels. However, Kv4.3/KChIP3 complex results to be more sensitive than Kv4.3 and Kv4.3/KChIP2c. Therefore, the sensitivity of Kv4.3 channels to CL888-like drugs will vary depending of the associated regulatory subunits. Granted by SAF2010-14916 and FIS-RIC RD12/0042/0019.

2754-Pos Board B446

Equilibrium Ion Binding Properties of Potassium-Selective and Non-Selective Cation Channels

Steve Lockless, Shian Liu.
Texas A&M University, College Station, TX, USA.

Selective cation transport across membranes is critical for rapid signaling in neurons and to setup ion gradients required for nutrient import. K^+ channels are a ubiquitous protein family that is highly selective for K^+ over Na^+ during ion conduction. They also have an inherent equilibrium preference for K^+ ions over Na^+ ions, suggesting that the equilibrium preference of a cation channel is important for its conduction selectivity. We present data showing K^+ channel variants that non-selectively conduct K^+ and Na^+ ions but who retain their equilibrium preference for K^+ ions. Based on this result, we propose a model of K^+ channel selectivity that relies on the architecture of the K^+ channel to translate the equilibrium preference into K^+-selective ion conduction (an inherently non-equilibrium process).

2755-Pos Board B447

Irreversible Binding of Ca^{2+} Channel β Subunit to α1B Revealed by Chemically-Inducible Dimerization System

Jun-Hee Yeon, Byung-Chang Suh.
Brain Science, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu, Korea, Republic of.

Voltage-gated calcium (Ca_V) channel β subunit increases the expression of pore-forming α1 subunit in the plasma membrane and regulates the biophysical properties of channel gating. In particular, the gating of high-voltage activated (HVA) Ca^{2+} channels is differentially controlled by Ca_V β subunits depending on the isotypes and intracellular location. However, the molecular mechanism of type specificity and cellular location of β subunit in Ca_V channel regulation is not clearly determined. We confirmed the constitutive localization of β2a in the plasma membrane irrelevant of the existence of α1B and α2δ1 is due to palmitoylation of N-terminus, and that $Ca_V2.2$ current with β2a is slowly inactivated compare to the cells with other types of cytosolic β subunits. In order to further understand the functional role of palmitoylation in the formation of channel complex and current regulation, we constructs a translocatable β2a(C3,4S)-FG a palmitoylation-resistant form of β2a, which is by tagged the β2a(C3,4S) with a FKBP domain and a green fluorescent protein (GFP) sequentially on its C-terminus as a chemically-inducible dimerization (CID) system. When the mutant β2a(C3,4S)-FG was expressed in tsA201 cells, it was mainly located in the plasma membrane in the presence of α1B, while it distributes throughout the cytosol without the α1B. Since the palmitoylation does not occur in the mutant form, the targeting of β2a(C3,4S)-FG to the plasma membrane can be only due to the interaction between α1B and β subunits. We also found that β2a(C3,4S)-FG and β2b-FG are not translocated to the cytosolic organelles by the application of rapamycin. Thus, the results demonstrate that the interaction between α1B and β2a subunits is irreversible.